SYNTHESIS OF BIOLOGICALLY ACTIVE PENTAPEPTIDE ANALOGS OF THE N-TERMINAL PART OF LIPOPROTEIN FROM THE OUTER MEMBRANE OF ESCHERICHIA COLI

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Newly synthesized lipopentapeptide derivatives with (R)-glycerol moieties showed higher mitogenic activities than those with the (S)-configuration.

KEYWORDS peptide synthesis; lipoprotein segment; mitogenic activity; chiral glycerol derivative; S-[2,3-bis(palmitoyloxy)propyl]-N-trichloroethoxycarbonyl pentapeptide

The lipoprotein from the outer membrane of Escherichia coli and other Enterobacteriaceae is a potent polyclonal activator for B lymphocytes. To determine the molecular structure responsible for the biological activities of lipoprotein, a series of oligopeptide analogs of its N-terminal part were synthesized. S-[2,3-bis(palmitoyloxy)-(2RS)-propyl]-N-palmitoyl-(R)-cysteiny1-(S)-seryl-(S)-seryl-(S)-asparaginy1-(S)-alanine was an active mitogen and polyclonal B lymphocyte activator in vitro and in vivo. It also supplements Salmonella vaccines. In this paper we describe a new synthesis of S-[2,3-bis(palmitoyloxy)-(2R and 2S)-propyl]-N-palmitoyl-(R)-cysteiny1-(S)-seryl-(S)-asparaginy1-(S)-alanine (1 and 3) and their N-(2,2,2-trichloroethoxycarbonyl)(2 and 4) by using the N-(2,2,2-trichloroethoxycarbonyl)cysteiny1 intermediates, which prevents the racemization of their cysteiny1 parts in the condensation steps.

\[
\begin{align*}
\text{CH}_2\text{OR} & \quad \text{CH}_2\text{OR} \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{RO} & \quad \text{RO} \\
\text{R = Cys-Ser-Ser-Asn-Ala-OH} & \quad \text{Troc = Cys-Ser-Ser-Asn-Ala-OH} \\
1 & \quad 2 \\
\text{CH}_2 & \quad \text{CH}_2 \\
\text{RO} & \quad \text{RO} \\
\text{R = CO(CH}_2\text{)}_{12}\text{CH}_3 & \quad \text{Troc = COOCH}_2\text{CCL}_3 \\
3 & \quad 4
\end{align*}
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The compounds 1, 2, 3 and 4 were synthesized according to the reaction sequence shown in Chart 1. The starting material 5 was prepared according to the method reported by K. H. Wiesmuller et al.\textsuperscript{23} N-protection of 5 with 2,2,2-trichloroethoxychloroformate (3 eq) in pyridine followed by reduction with di-thioretritol (4 eq) in CHCl\textsubscript{3}, in the presence of triethylamine (3 eq) afforded 7, which was used without further purification. Reaction of 7 with (R)-8\textsuperscript{19} in dimethylformamide in the presence of N,N-diisopropylethylamine (4 eq) gave 9 (55\% from 6). Esterification of 9 with palmitoyl chloride (2 eq) and N,N-diisopropylylethylamine (4 eq) in CH\textsubscript{2}Cl\textsubscript{2}, in the presence of a catalytic amount of 4-dimethylaminopyridine followed by deprotection of the tert-butyl group of 10 with trifluoroacetic acid afforded 11 in 69\% yield from (R)-8. Compound 13 was obtained in 61\% yield by coupling 11 with the pentapeptide 12\textsuperscript{20} in DMF using dicyclohexylcarbodiimide (1.1 eq) and 1-hydroxybenzotriazole (2 eq) as a coupling agent according to the method\textsuperscript{21} reported by K. H. Wiesmuller et al. Deprotection of all tert-butyl groups of 13 was carried out by treatment with trifluoroacetic acid to give 2\textsuperscript{10} in 45\% yield. The trichloroethoxy carbonyl group of 13 was removed by treatment with zinc in acetic acid to give 14, which was then acylated with palmitoyl chloride and N,N-diisopropylethylamine in CH\textsubscript{2}Cl\textsubscript{2} to afford 15. The final deprotection of all tert-butyl groups of 15 was carried out by treatment with trifluoroacetic acid to give 1\textsuperscript{11} (53\% yield from 13). In the same way the compounds 3\textsuperscript{12} and 4\textsuperscript{13} were synthesized by using (S)-8\textsuperscript{14} in place of (R)-8. The structures of 1, 2, 3 and 4 were supported by elemental analysis and confirmed by analysis of the IR, \textsuperscript{1}H-NMR and FAB/MS spectra. The chemical purity of 1, 2, 3 and 4 were determined to be 99.4\% (t\textsubscript{R}=4.31 min), 99.4\% (t\textsubscript{R}=4.36 min), 99.9\% (t\textsubscript{R}=4.28 min) and 99.4\% (t\textsubscript{R}=4.35 min) respectively by high performance liquid chromatography (HPLC) using an Asahipak column ODP-50 [0.6 x 15 cm, λ=210 nm, 0.1\% TFA/CH\textsubscript{3}CN \textasciitilde/\textasciitilde (7.5 min) + \textasciitilde/\textasciitilde\textasciitilde (12.5 min),

\[ \text{Chart 1} \]
flow rate 1.0 ml/min]. The mitogenic activities of all the lipopentapeptides 1, 2, 3 and 4 were measured. Compounds 1 and 4 had the same degree of activity and the activity of 2 was greatly enhanced. While the compound 3 activity was weak. These results indicate that the natural [(2R)-propyl] type 1 has a higher activity than the unnatural [(2S)-propyl] type 3 and that the Toc derivative increases mitogenic activity.

REFERENCES AND NOTES
8) (R)-8 was synthesized from (S)-1-O-tosyl-2-benzyl-glycerol\(^{155}\) in 65% yield, by deprotection of benzyl group (H\(_2\), Pd/C) and subsequent iodidation with NaI (3 eq) in a pressure bottle. mp 35-37 °C [\(\alpha\)\(_D\) = -6.0°(C=1.15 ,CHCl\(_3\)], IR (KBr): 3334 (OH).
9) 12 was synthesized from Z-Asn-Ala-OBu\(^{143}\) in the following steps [i. removal of Z-group (H\(_2\), Pd/C), ii. condensation of Z-Ser(Bu\(^{14}\)-OH and H-Asn-Ala-OBu\(^{14}\), iii. condensation of Z- Ser(Bu\(^{14}\)-OH and H-Ser(Bu\(^{14}\)-Asn-Ala-OBu\(^{14}\), iv. removal of Z-group (H\(_2\), Pd/C)]. which was identical with the corresponding authentic sample by Bessler\(^{144}\) in every respect (\(^{1}H\)-NMR, mp, RF, FABMASS).
10) mp 205-207 °C (white powder from CHCl\(_3\):MeOH=1:1), [\(\alpha\)\(_D\) =+9.20°(C=1.00 ,CHCl\(_3\)], FABMASS: m/z (M+H)* 1205, IR (KBr): 3300 (OH, NH), 1736 (O=C=O), 1662, 1537 (CONH).
11) mp 211-213 °C (white powder from CHCl\(_3\):MeOH=1:1), [\(\alpha\)\(_D\) =+56.5°(C=1.02 ,CHCl\(_3\)], FABMASS: m/z (M+H)* 1270, IR (KBr): 3284 (OH, NH), 1732 (O=C=O), 1627, 1550 (CONH).
12) mp 210-212 °C (white powder from CHCl\(_3\):MeOH=1:1), [\(\alpha\)\(_D\) =-28.3°(C= 0.86 ,CHCl\(_3\)], FABMASS: m/z (M+H)* 1270, IR (KBr): 3296 (OH, NH), 1736 (O=C=O), 1639, 1538 (CONH).
13) mp 204-207 °C (white powder from CHCl\(_3\):MeOH=1:1), [\(\alpha\)\(_D\) =+16.6°(C=1.00 ,CHCl\(_3\)], FABMASS: m/z (M+H)* 1205, IR (KBr): 3302 (OH, NH), 1737 (O=C=O), 1629, 1538 (CONH).
14) (S)-8 was synthesized starting from (S)-1-O-tosyl-2-benzyl glycerol in 51% yield in the following steps [i. protection with methoxymethyl chloride (1.2 eq), ii. deacetylation with NaOH, iii. tosylation with tosyl chloride, iv. demethoxymethylation with HCl, v. removal of benzyl group (H\(_2\), Pd/C), vi. iodidation with NaI in a pressure bottle.]. mp 35-37 °C, [\(\alpha\)\(_D\) = +6.0° (C=1.35, CHCl\(_3\)], IR: (KBr) 3334 (OH).

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